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Low-dose combination therapy of DUP-785 and RS-61443 prolongs cardiac allograft survival in rats

W. O. Bechstein*, Y. Suzuki, T. Kawamura, B. Jaffee, A. Allison, D. A. Hullett, and H. W. Sollinger

Department of Surgery, University of Wisconsin, Madison, Wisconsin, USA

The introduction of cyclosporine into the immunosuppressive armamentarium has revolutionized transplant surgery with significant improvements in graft survival. The apparent lack of effect of cyclosporine on humoral rejection mechanisms makes the search for other immunosuppressive agents desirable. Two anti-metabolites affecting nucleotide synthesis via different pathways have recently been evaluated for their immunosuppressive potential. DUP-785 (DUP), also known as brequinar sodium, reversibly inhibits de novo pyrimidine synthesis by blocking dihydro-orotate dehydrogenase, thus resulting in the delretion of critical precursors for RNA and DNA synthesis [2]. RS-61443, a morpholinoethyl ester of mycophenolic acid, reversibly and non-competitively blocks inosin monophosphate dehydrogenase, the key enzyme in purine de novo synthesis [10]. A possible additive effect of both drugs was investigated in the rat heart allograft model.

Key words: Immunosuppression – DUP-785 – RS-61443

Materials and methods

Heart grafts from male ACI ($RT1^a$) rats were transplanted into male Lewis (LEW) ($RT1^l$) rats in the heterotopic abdominal position using a modification of the technique described by Ono and Lindsey [9]. Syngeneic transplants between LEW rats served as controls. Drugs were applied by gavage, DUP 3 times weekly and RS daily. DUP was supplied in powder form as a gift from DuPont Merck, Wilmington, Del. RS was supplied as a gift from Syntex, Palo Alto, Calif. Graft function was monitored daily by abdominal palpation. Rejection was defined as the cessation of a palpable heart beat and confirmed by histology. Median graft survival between the groups was compared by the Mann-Whitney U-test. Recipients of syngeneic transplants were subjected to combination therapy with DUP and RS for a period of 30 days, whereafter they were sacrificed and any possible toxicity studied by histological evaluation of the lung, native and donor heart, stomach, small bowel, large bowel, kidney, pancreas, liver, and spleen specimens. Experimental groups included treatment with varying doses of DUP and RS alone or in combination (Table 1).

Results

Syngeneic transplants (group 0) survived for more than 100 days, whereas allogeneic transplants were rejected after a median of 6 days (group 1) (Table 1). Monotherapy with either DUP (3 or 6 mg/kg; groups 2, 3) or RS (10 mg/kg; group 4) in low doses led to a significant prolongation of graft survival; however, most transplants were rejected within 2 weeks. The addition of daily RS (10 mg/kg) to DUP (3 or 6 mg/kg, 3 times weekly; groups 5, 6) provided additional graft survival, and not only when compared with the control group; graft survival was significantly longer than in the respective groups treated by DUP alone (group 5 vs group 2, $P < 0.01$; group 6 vs. group 3 $P < 0.01$; Table 1).

Combination therapy with low doses of DUP and RS was well tolerated in all animals with no weight loss; ocasionally the animals would pass loose stools, but there was no frank diarrhea. Histological examination of tissue specimens in animals from the toxicity study who were sacrificed after 30 days of the combination therapy revealed no abnormalities except for 1 case each of slight gastric mucosal atrophy and some loss of large bowel mucosalepithelium in the DUP 6 mg/kg and RS 10 mg/kg combination therapy group.

Discussion

DUP-785 prevents cell proliferation by the inhibition of de novo pyrimidine biosynthesis [2]. It inhibits the development of delayed-type hypersensitivity to dinitrofluorobenzene (DNFB) in mice and prolongs heart, liver, and kidney allograft survival in the rat [3]. The effective

* *Present address:* Department of Surgery, University Clinic Rudolf Virchow, 1000 Berlin 65, Federal Republic of Germany

Offprint requests to: H. W. Sollinger, M. D., University of Wisconsin Hospital, H4/780 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792, USA

Table 1. Experimental groups and results of rat heterotopic heart grafting and therapy with DUP or RS alone and in combination

Experimental groups (graft survival time, days)	Median graft survival (days)	*P*-value (vs. group 1)
0. Syngeneic (LEW-LEW) $n = 10$ (79, 101 × 5,102 × 3,113)	> 100	
A. Immunosuppression		
1. Allogeneic (ACI-LEW) $n = 10$ no treatment	6	
2. (6, 6, 6, 6, 6, 6, 7, 7, 7, 8) Allogeneic $n = 10$ DUP 3 mg/kg	9.5	< 0.01
3. (8, 8, 8, 9, 9, 10, 11, 12, 13, 21) Allogeneic $n = 6$ DUP 6 mg/kg	14.5	< 0.01
4. (12, 12, 14, 15, 15, 19) Allogeneic $n = 10$ RS 10 mg/kg	8	< 0.01
5. (6, 7, 7, 7, 8, 8, 8, 8, 8, 10) Allogeneic $n = 10$ DUP 3 mg/kg + RS 10 mg/kg	19	< 0.001
6. (10, 11, 13, 13, 19, 19, 22, 33, 35, > 100) Allogeneic $n = 9$ DUP 6 mg/kg + RS 10 mg/kg	26	< 0.001
(19, 21, 23, 24, 26, 32, 34, 35, > 100)		
B. Toxicity		
7. Syngeneic $n = 4$ (4 × 31 days) DUP 3 mg/kg + RS 10 mg/kg		
8. Syngeneic $n = 4$ (4 × 31 days) DUP 6 mg/kg + RS 10 mg/kg		

Group 3 vs 2, $P < 0.05$; group 5 vs. 2, $P < 0.01$; group 6 vs. 3, $P < 0.01$; group 6 vs. 5, $P = 0.1$

dose for prolonging rat heart allograft survival for more than 30 days is 12–24 mg/kg 3 times weekly [3].

RS-61443 also blocks the proliferative responses of T and B lymphocytes and inhibits antibody formation and the generation of cytotoxic T cells [1, 4, 5, 8]. Furthermore, it prevents the rejection of pancreatic islet allograts in adult mice [6]. The effective dose for the prevention of heart allograft rejection in rats has been shown to be 30–40 mg/kg daily [7].

Since both drugs affect nucleic acid metabolism, they show major effects in rapidly dividing cell populations. Higher doses of both drugs lead to gastrointestinal symptoms such as diarrhea and ultimately weight loss.

In this study, we examined the effects of a combination therapy of low doses of DUP and RS on rat heart allograft survival. The dosages of the respective agents were at least half of what had previously been reported as effective. Furthermore, it was investigated whether combination therapy was feasible in terms of toxicity. Combination therapy with low doses of DUP and RS was well tolerated in all animals, with no weight loss and only the occasional occurrence of loose stools. Minor histological signs of toxicity like mucosal atrophy in the gastrointestinal tract appeared in 2 of 4 animals after 30 days of combination therapy with DUP 6 mg/kg and RS 10 mg/kg.

Low doses of RS and DUP alone significantly prolonged allograft heart survival in rats, but a combination therapy of DUP and RS in low doses proved to be even more powerful in regard to graft survival without increased toxicity. There is at least an additive effect of both drugs; further studies to investigate this synergism between both drugs are currently in progress.

DUP inhibits the pathway of pyrimidine synthesis at a relatively early step, while RS interferes with purine de novo synthesis at a rather late stage. It may be hypothesized that treatment with DUP renders expanding lymphocyte populations more susceptible to the effects of RS treatment without any further increase in toxicity.

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